EZ-Triglyceride Quantification Assay Kit

Metabolism assay kit

(Colorimetric/Fluorometric)

Cat. No. DG-TGC100

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

DoGenBio Co., Ltd.

▪ Product Description

Triglyceride (TG) is a major component of vegetable oil, animal fat, LDL, and VLDL, and plays an important role as an energy source or as a carrier of fatty acids. TG is broken down into fatty acids and glycerol, and can later act as a substrate for energy production and metabolic pathways. Hypertension caused by TG is related to atherosclerosis, heart disease, and stroke as well as pancreatitis.

EZ-Triglyceride Quantification Assay Kit provides a sensitive and easy analytical method to measure the concentration of triglycerides, monoglycerides and diglycerides in various samples. In the analysis, TG is decomposed into free fatty acids and glycerol. Then, after oxidizing glycerol, the byproduct (H_2O_2) formed reacts with the probe to generate absorbance (O.D. 570 nm) and fluorescence (Ex/Em = 535/590 nm).

▪ Contents and Storage Conditions

Component	100 assay	Cap Cord	Storage
Triglyceride Assay buffer	25㎖	-	-20℃
Triglyceride Enzyme mix (Lyophilized)	1 vial	Red	-20℃
Lipase (Lyophilized)	1 vial	Blue	-20℃
Triglyceride Probe	200㎕	Yellow	-20℃
Triglyceride Standard (1 mM)	300㎕	Green	-20℃

* This product is for research use only and is not intended for human or diagnostic use.

* In terms of the number of tests that can be performed with this product, 100 assays means that it provides reagents that can process 100 wells based on 1 well of a 96 well plate. Among these, considering standard, blank, duplication processing per sample, etc., the actual number of samples that can be tested is in the range of 20 to 40 samples. Review the product instructions carefully and determine the number of kits required considering the characteristics of the sample you wish to test.

▪ Sample type

Triglyceride concentrations can be measured in samples such as animal tissues, cultured cells (adipocytes), serum or plasma, and thus lipid metabolism can be analyzed.

▪ Preparation of Reagent

Component	Preparation	Storage and Stability
Triglyceride Enzyme mix (Lyophilized)	Add 220 ㎕ Assay Buffer and mix well using a pipette.	The mixed solution is stable for 2 months at -20℃.
Lipase (Lyophilized)	Add 220 ㎕ Assay Buffer and mix well using a pipette.	The mixed solution is stable for 2 months at -20℃.
Triglyceride Probe	Use after sufficiently dissolving at room temperature.	The solution can be stored at -20℃, but it is recommended to use it within 2 months.
Triglyceride Standard	The aqueous phase may separate when stored frozen. Before use, melt at 37℃ for 5 minutes and vortex. Make sure the solution is transparent and the triglyceride is completely mixed.	The solution can be stored at -20℃, but it is recommended to use it within 2 months.

▪ General Protocol

1. Sample preparation

Add 2-50 ㎕ of the prepared sample to a 96-well plate, and adjust the final volume to 50 ㎕ with assay buffer. (n≥2)

1) Serum

Proceed with analysis immediately without preparation.

2) Cell or Tissue (non-aqueous samples)

① Prepare ~ 1 x 10^6 cells or ~ 100 mg tissue sample.
② Wash the sample using PBS.
③ Add 1 ml of 5% NP-40 (in D.W.) to the sample and homogenize on ice.
④ Heat at 80-100℃ for 2-5 minutes or until the solution becomes opaque, then cool sufficiently at room temperature.
⑤ Heat once more to dissolve all triglycerides.
⑥ Centrifuge at 10,000xg for 2 minutes, remove insoluble substances, and dilute the sample 10-fold with distilled water before analysis.

3) For unknown samples or samples being measured for the first time, it is recommended to conduct a preliminary experiment to ensure that the measured values are within the standard curve before use.

4) For samples with high background, prepare the same amount of sample used for measurement as a background control.

5) Because endogenous compounds can interfere with the reaction, a spike test is recommended to accurately measure triglycerides by adding a certain amount (4 nmol) of triglyceride standard to the sample.

2. Standard preparation

1) Colorimetric method

Add 0, 2, 4, 6, 8, and 10 ㎕ of 1 mM triglyceride standard into a 96-well plate and adjust the final volume to 50 ㎕ with assay buffer to create standard sets of 0, 2, 4, 6, 8, and 10 nmol/well for each plate.

Standard No.	Volume of 1mM Triglyceride Standard	Volume of Assay buffer	Final standard volume in well	Final standard Triglyceride Conc. (nmol/well)
1	0 ㎕	50 ㎕	50 ㎕	0
2	2 ㎕	48 ㎕	50 ㎕	2
3	4 ㎕	46 ㎕	50 ㎕	4
4	6 ㎕	44 ㎕	50 ㎕	6
5	8 ㎕	42 ㎕	50 ㎕	8
6	10 ㎕	40 ㎕	50 ㎕	10

* For accurate measurement, it is recommended to prepare and conduct experiments with more than two replicates each of standards and samples.

* Standard recommends measurement during each experiment.

2) Fluorometric method

Prepare a 0.1 mM standard solution by mixing 10 ㎕ of 1 mM triglyceride standard and 90 ㎕ of assay buffer, and dispense 0, 2, 4, 6, 8, and 10 ㎕ into each well. Adjust the final volume to 50 ㎕ with assay buffer, and standard sets of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 nmol/well will be created for each plate.

Standard No.	Volume of 0.1mM Triglyceride Standard	Volume of Assay buffer	Final standard volume in well	Final standard Triglyceride Conc. (nmol/well)
1	0 ㎕	50 ㎕	50 ㎕	0
2	2 ㎕	48 ㎕	50 ㎕	0.2
3	4 ㎕	46 ㎕	50 ㎕	0.4
4	6 ㎕	44 ㎕	50 ㎕	0.6
5	8 ㎕	42 ㎕	50 ㎕	0.8
6	10 ㎕	40 ㎕	50 ㎕	1.0

* For accurate measurement, it is recommended to prepare and conduct experiments with more than two replicates each of standards and samples.

* Standard recommends measurement during each experiment.

3. Lipase

Add 2 ㎕ of Lipase to each standard and sample well and incubate at room temperature for 20 minutes.

* Do not add Lipase to the background control wells, but add 2㎕ of Assay buffer.

4. Reaction mixture preparation

This is the volume based on 1 assay. Calculate the amount of assay you want to use in the experiment and prepare the reaction mix with enough volume.

1) Colorimetric method

Components	Reaction mixture
Triglyceride Assay buffer	46㎕
Triglyceride Enzyme mix	2㎕
Triglyceride Probe	2㎕
Total	50㎕

2) Fluorometric method

Components	Reaction mixture
Triglyceride Assay buffer	47.6㎕
Triglyceride Enzyme mix	2㎕
Triglyceride Probe	0.4㎕
Total	50㎕

5. Add 50 ㎕ of the reaction mixture to each well containing the triglyceride standard and sample using a multi pipette and mix well.

* If you have prepared a background control, add the background mixture to the background control well.

6. After incubation the plate at room temperature with light blocked for 30 minutes, gently shake it and measure it with a microplate reader.

1) Colorimetric : 570 nm

2) Fluorometric : (Excitation/Emission): 535 nm / 595 nm

▪ Calculation

1. Subtract the standard 1 value (blank) from all measurements.

2. Average duplicate measurements from each standard well and sample well.

3. Determine the standard curve using the triglyceride standard absorbance.

4. The amount of triglyceride in the sample is calculated by substituting the sample measurement values into the standard curve.

* If background control is set, the amount of triglyceride is calculated by subtracting the background control measurement value from the sample measurement value.

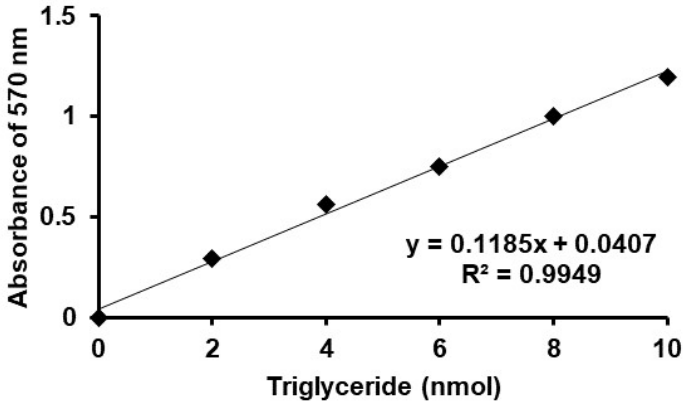
5. Based on the amount of triglyceride in the sample calculated in 4, the concentration of cholesterol in the sample is calculated using the following formula.

Triglyceride concentration of sample (C) = B/V x D (nmol/㎕ or mM)

B : Amount of cholesterol in the measurement well obtained from the standard curve (nmol)

V : Amount of sample in a well (㎕)

D : Sample dilution ratio (if diluted twice, calculate as x2, not x1/2)



Triglyceride standard curve. Assay was performed following the kit protocol.

※ Spike sample : If there is a possibility that some component in the sample may have affected the reaction, for example, there may be cases where the result shows that only 1.6 ng (80%) of triglyceride is present even though there is actually 2 ng of triglyceride due to the influence of some other substance. To correct this phenomenon, a separate well is set up with a certain amount of triglyceride (using a standard substance) added to the sample, and the concentration of the actual sample is corrected through the result value. If a spike sample is used in this experiment, the above concentration calculation formula is summarized as follows.

샘플 내 Triglyceride 의 양(B) = OD1 / (OD2 – OD3) x Triglyceride spike (nmol)

OD1 : OD value of Sample (blank corrected)
OD2 : OD value of Spiked sample (blank corrected)
OD3 : OD value of Sample (blank corrected)
Triglyceride spike : Amount of Triglyceride spike added to the sample
(*Triglyceride molecular weight : 885.4 g/mol)

▪ Related Product

	Products	Catalog No.	Assay
Oxidative Stress Assay Kit	EZ-Superoxide Dismutase (SOD) Assay Kit (Colorimetric)	DG-SOD400	400 Assay
	EZ-Glutathione Assay Kit (Colorimetric)	DG-GLU200	200 Assay
	EZ-Catalase Assay Kit (Fluorometric/Colorimetric)	DG-CAT400	400 Assay
	EZ-Hydrogen peroxide/Peroxidase Assay Kit (Fluorometric/Colorimetric)	DG-PER500	500 Assay
	EZ-Lipid Peroxidation (TBARS) Assay Kit (Colorimetric)	DG-TBA200	200 Assay
	EZ-Total Antioxidant Capacity (TAC) Assay Kit (Colorimetric)	DG-TAC200	200 Assay
	EZ-DPPH Antioxidant Assay Kit (Colorimetric)	DG-DPH400	400 Assay
	EZ-ABTS Antioxidant Assay Kit (Colorimetric)	DG-ABT400	400 Assay
	EZ-Glutathione Peroxidase Assay Kit (Colorimetric)	DG-GPX100	100 Assay
Metabolism Assay Kit	EZ-Lactate Assay Kit (Colorimetric)	DG-LAC100	100 Assay
	EZ-Acetylcholinesterase Assay Kit (Colorimetric)	DG-ACE100	100 Assay
	EZ-Ascorbic Acid Assay Kit (Colorimetric)	DG-ASC100	100 Assay
	EZ-ATP Assay Kit (Fluorometric/Colorimetric)	DG-ATP100	100 Assay
	EZ-Free Fatty Acid Assay Kit (Fluorometric/Colorimetric)	DG-FFA100	100 Assay
	EZ-Free Glycerol Assay Kit (Fluorometric/Colorimetric)	DG-FGC100	100 Assay
	EZ-Glucose Assay Kit (Fluorometric/Colorimetric)	DG-GCS100	100 Assay
	EZ-HDL, LDL/VDL Assay Kit (Fluorometric/Colorimetric)	DG-CHO100	100 Assay
	EZ-Total Cholesterol Assay Kit (Fluorometric/Colorimetric)	DG-TSC100	100 Assay
	EZ-Triglyceride Quantification Assay Kit (Fluorometric/Colorimetric)	DG-TGC100	100 Assay
	EZ-Nitric Oxide Assay kit (Colorimetric)	DG-NO500	500 Assay
	EZ-Total Collagen Assay Kit (Colorimetric)	DG-COL100	100 Assay
	EZ-Ethanol Assay Kit (Colorimetric)	DG-ETH100	100 Assay